CLINICAL RESEARCH ARTICLE

See Editorial on pages 191-192 in this issue.

MUSCLE&NERVE WILEY

Body mass index associates with amyotrophic lateral sclerosis survival and metabolomic profiles

Stephen A. Goutman MD, MS [1,2] | Jonathan Boss MS [3] | Gayatri Iyer MS [4] |
Hani Habra PhD [4] | Masha G. Savelieff PhD [2] | Alla Karnovsky PhD [4] |
Bhramar Mukherjee PhD [3] | Eva L. Feldman MD, PhD [1,2]

[1]Department of Neurology, University of Michigan, Ann Arbor, Michigan, USA

[2]NeuroNetwork for Emerging Therapies, University of Michigan, Ann Arbor, Michigan, USA

[3]Department of Biostatistics, University of Michigan, Ann Arbor, Michigan, USA

[4]Department of Computational Medicine & Bioinformatics, University of Michigan, Ann Arbor, Michigan, USA

Correspondence
Stephen A. Goutman, MD, MS, Department of Neurology, 1500 E Medical Center Dr, Ann Arbor, MI 48109-5223, USA.
Email: sgoutman@med.umich.edu

Funding information
Centers for Disease Control and Prevention, Grant/Award Numbers: 200-2013-56856, R01TS000289; National Center for Advancing Translational Sciences, Grant/Award Number: UL1TR002240; National Institute of Environmental Health Sciences, Grant/Award Numbers: K23ES027221, R01ES030049; National Institute of Neurological Disorders and Stroke, Grant/Award Number: R01NS127188; NeuroNetwork for Emerging Therapies; NeuroNetwork Therapeutic Discovery Fund; Peter R. Clark Fund for ALS Research; Scott L. Pranger; Sinai Medical Staff Foundation

Abstract

Introduction/Aims: Body mass index (BMI) is linked to amyotrophic lateral sclerosis (ALS) risk and prognosis, but additional research is needed. The aim of this study was to identify whether and when historical changes in BMI occurred in ALS participants, how these longer term trajectories associated with survival, and whether metabolomic profiles provided insight into potential mechanisms.

Methods: ALS and control participants self-reported body height and weight 10 (reference) and 5 years earlier, and at study entry (diagnosis for ALS participants). Generalized estimating equations evaluated differences in BMI trajectories between cases and controls. ALS survival was evaluated by BMI trajectory group using accelerated failure time models. BMI trajectories and survival associations were explored using published metabolomic profiling and correlation networks.

Results: Ten-year BMI trends differed between ALS and controls, with BMI loss in the 5 years before diagnosis despite BMI gains 10 to 5 years beforehand in both groups. An overall 10-year drop in BMI associated with a 27.1% decrease in ALS survival ($P = .010$). Metabolomic networks in ALS participants showed dysregulation in sphingomyelin, bile acid, and plasmalogen subpathways.

Discussion: ALS participants lost weight in the 5-year period before enrollment. BMI trajectories had three distinct groups and the group with significant weight loss in the past 10 years had the worst survival. Participants with a high BMI and increase in weight in the 10 years before symptom onset also had shorter survival. Certain metabolomics profiles were associated with the BMI trajectories. Replicating these findings in prospective cohorts is warranted.

KEYWORDS
amyotrophic lateral sclerosis, body mass index, metabolism, prognosis, survival

---

---

1 | INTRODUCTION

Amyotrophic lateral sclerosis (ALS) diagnosis is preceded by a presymptomatic phase, characterized by initiation of the disease process but lacking pronounced clinical symptoms.[1–3] ALS patients frequently experience a rapid decrease in body mass index (BMI) and the rate of loss early in the disease course is a strong prognostic factor.[4] Therefore, BMI loss may reflect an early and presymptomatic manifestation of disease. Indeed, individuals with ALS develop BMI loss many years before symptom onset.[5] Additionally, lower BMI earlier in life may both increase ALS risk[5–9] and decrease ALS survival.[5,10]

BMI decreases in ALS patients are linked to lower energy intake from dysphagia and higher energy expenditure,[11,12] including hypermetabolism, altered glucose and lipid metabolism, and mitochondrial dysfunction.[13] Perturbations in metabolism in ALS are supported by correlations in basic lipid profiles with risk and outcomes. Increased low-density lipoprotein cholesterol (LDL-C) and apolipoprotein B levels years before ALS diagnosis are associated with a higher future risk of ALS onset[14]; higher levels of both at diagnosis also associated with a lower risk of death.[15] However, basic lipid profiles do not capture the full spectrum of metabolic changes that occur in disease. Rather, the metabolome and lipidome, the cumulative profile of all metabolites and lipids, may more comprehensively reflect the metabolic state. Indeed, metabolomics profiles correlate with BMI[16–18] and disease phenotypes, such as cardiometabolic risk.[16,17] In the future, metabolomics signatures may be useful in combination with BMI as predictors of disease outcomes.[16]

However, the correlation of BMI with metabolomics profile and disease outcomes has not been investigated in ALS. Thus, our goal in this study was to leverage our case/control study to examine trends in BMI trajectory in ALS vs control participants correlated with survival and metabolomics profile.

2 | METHODS

2.1 | Participants and samples

Recruitment and data collection procedures are published.[19–22] Briefly, all patients seen at the Pranger ALS Clinic at University of Michigan with an ALS diagnosis, age at least 18 years, and ability to consent in English were asked to participate. Neurologically healthy controls, recruited through population outreach, completed the same procedures. All participants provided oral and written informed consent and the study was approved by the institutional review board of the University of Michigan. Demographic characteristics and available previous heights and weights from the medical records of the participants were obtained, as were ALS disease characteristics according to the Revised El Escorial criteria (rEEC).[23] Participants were asked to self-report height in feet and inches and weight in pounds 10 years ago, 5 years ago, and at the present time. For ALS participants, present weight was typically equivalent to weight at diagnosis since enrollment occurred shortly after diagnosis. BMI was calculated from height and weight as follows: weight (kg) / [height (m)]^2.[24] ALS participants with an interval of more than 5 years from symptom onset to diagnosis were not included in the analysis as the goal was to investigate presymptomatic differences in BMI. A subset of participants provided plasma for metabolomics analysis, as described elsewhere.[25,26]

2.2 | Descriptive analysis

Descriptive statistics were calculated for demographic characteristics including age, sex, ALS disease-onset segment frequencies, and disease duration (time from symptom onset to diagnosis). Study population differences were compared between BMI groups by analysis-of-variance and chi-square tests. Lin's concordance correlation coefficient quantified agreement between available self-reported and measured BMIs.

2.3 | BMI progression analysis and group assignment

Generalized estimating equations (GEEs) with unstructured correlation structure assessed differences in BMI changes for ALS and control participants, while accounting for within-participant correlation between self-reported BMI measurements.[27] The GEE outcome was self-reported BMI and the covariates were interaction terms between ALS/control status and the three time-points adjusted for age and sex at study entry. Differences in average BMI rate of change between ALS and controls were assessed using the Wald test and performed with the R geepack package (R Foundation for Statistical Computing, Vienna, Austria).[28]

After subtracting self-reported BMI 10 years before consent from all time-points, k-means clustering for longitudinal data (kml R package[29]) grouped ALS cases based on their self-reported changes in BMI, for use in ALS survival models. This subtraction step ensured that the k-means procedure clustered exclusively on BMI changes over time, rather than differences in baseline BMI. After considering two to six clusters, the selected number of clusters maximized the Calinski and Harabasz criterion,[30] a measure of between-cluster variation relative to within-cluster variation for longitudinal data.[31] The distance metric used for clustering was Euclidean distance with Gower adjustment.[31]

2.4 | Survival analysis

Kaplan-Meier plots of survival from diagnosis by cluster were produced. Cox proportional hazards models determined associations between cluster groups and ALS survival, defined as the time from diagnosis to death. Associations were adjusted for sex, age, baseline BMI (ie, 10 years earlier), onset segment, diagnosis rEEC, and time from symptom onset to diagnosis. Proportional hazards assumptions were checked using global and individual Schoenfeld tests with graphical assessment of the rescaled Schoenfeld residuals over time. Due to

proportional hazards violations in some models, accelerated failure time (AFT) models were constructed.

2.5 | Sensitivity analyses

Sensitivity analyses for incomplete BMI data (inverse probability weighted models) and nonlinear effects of BMI were performed (Supplemental Methods). $P < .05$ was considered statistically significant for the analyses.

2.6 | Metabolomics data analysis

Plasma samples from ALS participants were analyzed by Metabolon (Morrisville, North Carolina), with data published previously in case-control analyses.[25,26] Plasma samples were nonfasting as this was considered unethical for many ALS participants. Metabolomics analysis included data set normalization, computing correlations between BMI and metabolites, and selecting metabolites associated with BMI trajectory via least absolute shrinkage and selection operator (lasso) regression (Supplemental Methods). To identify highly interconnected metabolic modules, further analysis included construction of a partial correlation network using a previously published sparse partial correlation algorithm,[32–34] followed by consensus clustering.[35] Finally, group-penalized lasso regression (group lasso) models were created to identify metabolic modules associated with BMI clusters (Supplemental Methods). Group lasso[36] is a generalization of lasso regression, which has the advantage of incorporating before information on the grouping structure of the covariates, that is, the metabolic modules in this instance. Analyses were performed with R version 4.0.2.

3 | RESULTS

3.1 | Participants

For those with BMIs observed at all three time-points, ALS participants represented a typical patient population, according to onset age, distribution of segment onset, among other variables. Controls (n = 266) were slightly younger than cases (n = 381) (Table 1). Two ALS participants with an uncertain onset segment and one control with a BMI greater than 100 kg/m^2 labeled as an outlier were removed from subsequent analysis.

3.2 | BMI trends in cases vs controls

Lin's concordance correlation coefficient showed consistency between self-reported and measured BMI values (Supplemental Results). ALS and control participants reported BMI increases in the

TABLE 1 Participants' demographics

Covariate	Overall (n = 647)	ALS cases (n = 381)	Controls (n = 266)	P value
Mean age at survey consent, years	63.3 (56.5-69.9)	64.9 (57.6-71.4)	61.3 (55.2-68.2)	<0.001
Sex				0.143
Female	317 (49.0)	177 (46.5)	140 (52.6)	
Male	330 (51.0)	204 (53.5)	126 (47.4)	
Last contact event				NA
Death		251 (64.9)	NA	
Censored		130 (34.1)	NA	
Original and/or revised El Escorial criteria				NA
Possible/suspected		53 (13.9)	NA	
Probable, LS		104 (27.3)	NA	
Probable		123 (32.3)	NA	
Definite		101 (26.5)	NA	
Onset segment				NA
Bulbar		113 (29.7)	NA	
Cervical		126 (33.1)	NA	
Lumbar		142 (37.3)	NA	
Time between symptom onset and diagnosis (years)		1.01 (0.64-1.66)	NA	NA

Note: For continuous variables, data expressed as median (25th to 75th percentile); for categorical variables, data expressed as number (%). *P* values for continuous and categorical variables correspond to analysis of variance and chi-square tests, respectively.

Abbreviations: ALS, amyotrophic lateral sclerosis; LS, laboratory supported; NA, not applicable.

TABLE 2 Accelerated failure time model

	Percent change in survival	LCL	UCL	P value
Age at entry (years)	−1.0	−1.9	−0.2	0.016
Symptom onset to diagnosis (log years)	17.3	3.3	33.2	0.014
Baseline BMI	−1.0	−2.7	0.8	0.278
Decrease BMI trajectory	−27.1	−42.6	−7.3	0.010
Increase BMI trajectory	−7.1	−25.2	15.5	0.509
Male	0.1	−16.1	19.4	0.994
Cervical onset	41.0	13.0	76.0	0.002
Lumbar onset	21.3	−1.4	49.3	0.068
rEEC possible/suspected	88.3	41.9	149.7	0.000
rEEC probable	23.4	−0.7	53.3	0.058
rEEC probable, laboratory supported	61.6	28.5	103.1	0.000

Abbreviations: BMI, body mass index; LCL, lower confidence limit; rEEC, revised El Escorial criteria; UCL, upper confidence limit.

10- to 5-year period before study entry (Figure S1). Unlike controls, however, ALS cases had an overall BMI decrease in the 5-year prior-to-study entry time window. The age- and sex-adjusted GEE model showed average ALS BMI change from −5 years to 0 year was 1.75 kg/m^2 (95% confidence interval [CI], 1.35 to 2.16 kg/m^2; $P < 1 \times 10^{-17}$), but was only 0.02 kg/m^2 for controls (95% CI, −0.35 to 0.40 kg/m^2; $P = .9$). Thus, ALS participants report BMI loss occurring 5 years before diagnosis/study entry, whereas control participants had no significant BMI change during the same time-frame. The kml algorithm applied to the ALS participant BMI trajectories identified three clusters, defined as decrease, mild decrease, and increase BMI groups (Supplemental Results, Figure S2, and Table S1).

3.3 | Survival analysis

Unadjusted Kaplan-Meier survival analysis showed decreased absolute median survival times for the decrease BMI cluster (Figure S3). Some Cox models violated proportional hazards by Schoenfeld residuals, so AFT models were constructed. After adjusting for age, sex, baseline BMI (ie, 10 years earlier), onset segment, rEEC, and time from symptom onset to diagnosis, participants in the decrease BMI cluster had a 27.1% shorter survival (95% CI, −42.6% to −7.3%; $P = .010$) vs the mild decrease group (Table 2 and Figure 1). Results were similar in missing BMI data sensitivity analyses and when using base BMI as a categorical variable (see Supplemental Results, Figures S4 and S5, and Tables S2, S3, S4, and S5). Interestingly, in sensitivity analyses for interaction effects between baseline BMI and change in BMI over time, ALS participants with an obese baseline BMI and increase BMI trajectory had shorter survival, similar to participants in the decrease BMI trajectory group (Table S5).

3.4 | Metabolites associated with BMI trajectory

Metabolomic differences by BMI cluster (decrease, mild decrease, increase) were investigated for the 207 participants with available
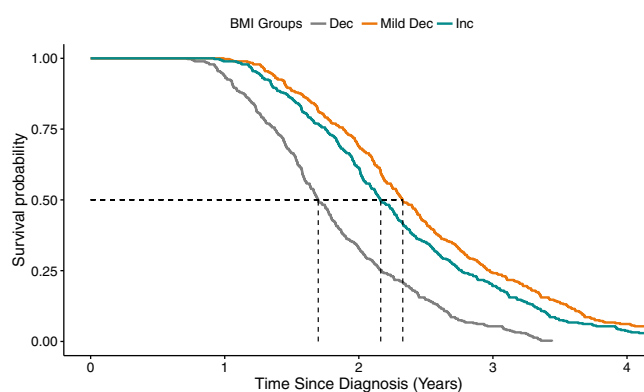

FIGURE 1 Accelerated failure time model plots. Covariate adjusted survival curves corresponding to the unweighted accelerated failure time model with body mass index (BMI) cluster groups. The estimated median survival time is 1.7 years for the decrease BMI group, 2.33 years for the mild decrease BMI group, and 2.16 years for the increase BMI group. Dec, decrease; Mild dec, mild decrease; Inc, increase.

previously published untargeted metabolomics.[25,26] The final curated data set included 607 metabolites from plasma collected near the time of diagnosis. Associations of individual metabolites with BMI trajectory groups are presented in the Supplemental Results and Tables S6 and S7.

The partial correlation network was constructed using recently published data from 349 ALS participants,[26] of whom 207 were also in this analysis. Including additional samples generated a more informative network because partial correlation methods are sensitive to sample size. The resulting partial correlation network contained 600 metabolites connected by 887 edges (false discovery rate–adjusted $P < .1$), of which 31 had a negative partial correlation coefficient. Seven metabolites did not have any significant correlations and were not included in the network. Consensus clustering identified 26 metabolic modules spanning 555 highly connected metabolites. The remaining 45 metabolites did not
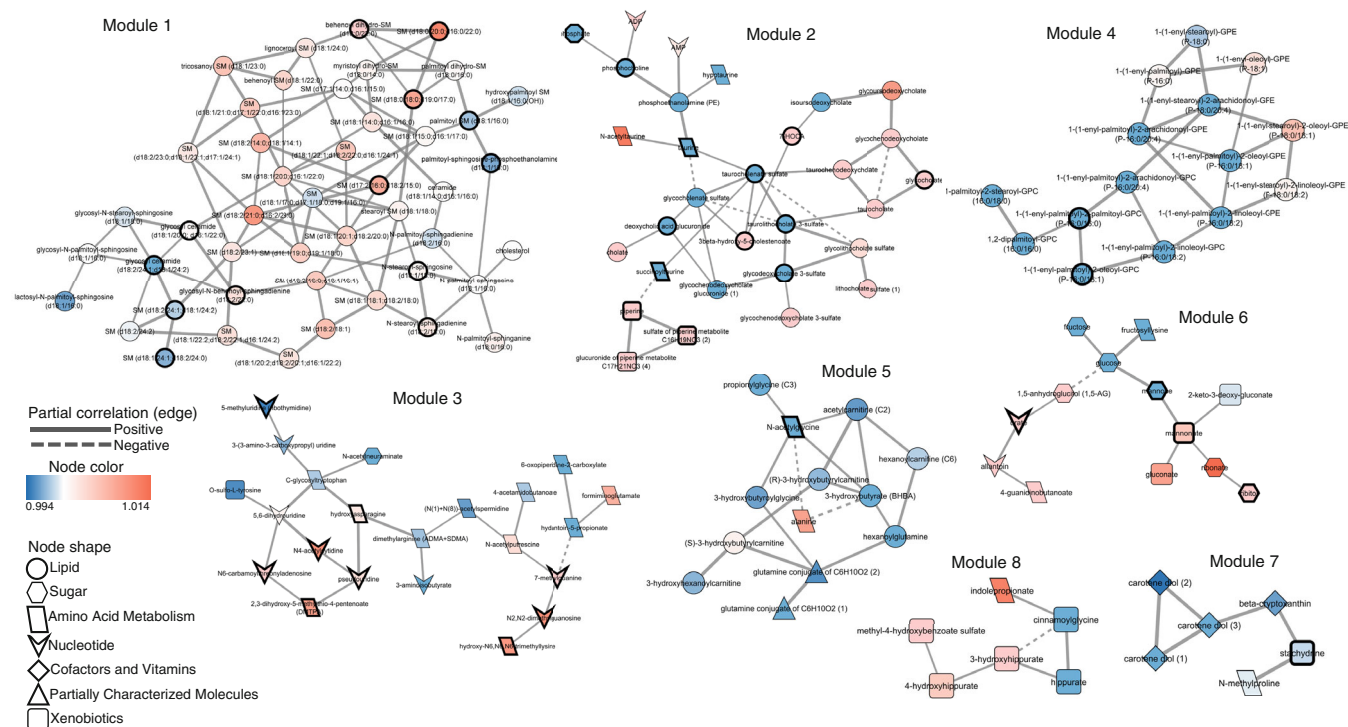
FIGURE 2   Metabolic modules associated with body mass index (BMI) trajectory. Eight metabolic modules containing 152 total metabolites associated with BMI trajectory in group lasso regression models. Node color indicates odds ratio (OR) from group lasso: OR > 1 indicates association with the increase BMI cluster (red node), OR < 1 indicates association with the decrease BMI cluster (blue node). Nodes with a bold border correlate significantly with current BMI (false discovery rate < 0.05). Node shape indicates the subpathway to which the metabolite belongs. Solid edge between metabolites indicates positive partial correlation coefficient. Dashed edge indicates negative partial correlation coefficient.

Metabolic module	Number of nodes (metabolites)	Number of edges	Average degree[a]	Metabolic pathways
1	47	88	3.76	Ceramides, sphingomyelins
2	30	41	1.367	Bile acid metabolism, amino acid and purine metabolism
3	22	23	2.09	Amino acid, nucleotide metabolism
4	15	21	2.8	Plasmalogens, lyso-plasmalogens, phosphatidylcholines
5	13	18	2.77	Fatty acid metabolism (acyl carnitines, acyl amino acids)
6	13	12	1.85	Carbohydrate, amino acid, nucleotide metabolism
7	6	6	2	Vitamin A metabolism, amino acid metabolism
8	6	6	2	Benzoate metabolism, amino acid metabolism

TABLE 3   Metabolomics modules from group lasso associate with BMI trajectory groups

[a]Average degree represents the average number of connections each node (metabolite) makes within the module and indicates the network/module density.

cluster due to poor connectivity. Metabolic module size ranged from 5 to 66 metabolites (Figure 2).

Group lasso selected eight modules containing 152 metabolites, which associated with the decrease and increase BMI clusters (Figure 2, Table 3, and Table S8), with odds ratios (ORs) ranging from

0.92 to 1.1 (Table S9). The largest module 1 (47 metabolites) included ceramides and sphingomyelins, of which 36 had OR > 1, indicating associations with the increase BMI cluster. The second largest module 2 (30 metabolites) included primary and secondary bile acid metabolites, taurine and its derivatives, AMP, ADP, and sterols. Primary bile

acids associated with the increase BMI cluster (OR > 1), whereas most secondary bile acids and taurine metabolites associated with the decrease BMI cluster (OR < 1). Module 3 (22 metabolites) primarily contained amino acid and nucleotide metabolites, half of which associated with the decrease BMI cluster. Module 4 (15 metabolites) was composed of plasmalogens, lyso-plasmalogens, and phosphatidylcholines, 11 of which associated with the decrease BMI cluster. Module 5 (13 metabolites) had mostly acyl carnitines, acyl amino acids, and some other amino acid metabolites, which mostly associated with the decrease BMI cluster. The remaining smaller module 6 (13 metabolites; sugar and nucleotide metabolites, xenobiotics, amino-sugar), module 7 and module 8 (6 metabolites each; xenobiotics, cofactors, vitamins, modified amino acids) contained various metabolites.

Overall, these results suggest that unique metabolomic profiles correlate with BMI trends in participants with ALS, especially metabolism centered on ceramides, sphingomyelins, and primary and secondary bile acids.

4 | DISCUSSION

This study adds to the growing body of evidence that presymptomatic BMI loss is linked to ALS risk and survival. We have shown that ALS participants are characterized by significant BMI loss at 5 years, but not 10 years, before study entry as compared with control participants. A decrease in BMI trajectory was associated with shorter survival in ALS, which also correlated with a distinct metabolomic profile. Our study also suggests that BMI loss may occur during the presymptomatic phase of ALS leading up to diagnosis. Several other studies have similarly shown BMI decrease preceding ALS diagnosis, out to 10 years before onset,[5] and even within the decades preceding ALS.[6,9] Although we found BMI trajectories differed over the 10-year window, absolute BMI did not vary between ALS and control participants 10 years before study entry when participants would have had a mean age of 54.9 (ALS) and 51.3 (controls) years. In contrast, other studies reported that lower mid- to late-life BMI increases ALS risk,[8,9,37] although one study reported ALS survival depends on BMI change, not on BMI before or at diagnosis.[4] Another recent study has suggested that BMI in ALS patients diverges from controls 10 years before disease onset.[38]

Next, we found that that ALS participants with a 10-year decrease BMI trend had shorter survival. Our results are consistent with several studies demonstrating that a drop in BMI before ALS diagnosis correlates with poorer survival.[4,5,9,39] In particular, an analysis of the Piemonte and Valle d'Aosta Register for ALS showed that BMI loss at diagnosis was more prognostic of survival than BMI either before or at diagnosis.[4] However, because there is literature showing that BMI is an ALS risk factor,[8,9,37] we conducted sensitivity analyses to assess the interaction of baseline BMI with BMI trajectory. We found that normal baseline BMI lengthened survival in the decrease BMI trajectory group, whereas obese baseline BMI shortened survival in the increase BMI trajectory group. Baseline BMI only marginally influenced survival in the mild decrease BMI trajectory group.

Interestingly, the European Prospective Investigation into Cancer and Nutrition study also showed that obese females had shorter survival, which did not reach significance,[22] but the Piemonte and Valle d'Aosta Register showed no impact of BMI on survival.[4]

The reasons for survival differences by BMI or BMI change in ALS are not known. However, the prevailing theories are related to impaired energy homeostasis,[11] with lowered energy intake fighting against higher energy expenditure. Dysphagia is a frequent cause of reduced energy intake; however, in ALS, BMI loss also occurs independent of dysphagia,[4,39] indicating the presence of significantly elevated energy expenditure. Indeed, one study showed that hypermetabolism was more frequent in ALS than in control participants and correlated inversely with survival.[12] Resting energy expenditure may additionally interact with BMI and fat mass to influence survival in ALS.[40,41]

In our study, we employed data-driven network analysis to identify highly interconnected metabolic modules and we assessed their correlation with BMI trajectory groups. The largest of these, module 1, contained ceramides (13 species) and sphingomyelins (33 species). The latter were primarily associated with the increase BMI group. In earlier work, we and others found that sphingomyelins also differ in analyses of ALS vs control participant plasma.[25,26,42–45] Further, one recent study reported that higher sphingomyelin levels may correlate with faster disease progression.[45] Sphingomyelins are a large class of lipids that have structural roles in cell membranes and lipid rafts, and, through hydrolysis to ceramides, with signaling activity, for example, pro-apoptotic, excitotoxic, neurotoxic.[46,47] Impaired sphingomyelin metabolism may be an integral factor in ALS as supported by investigations of genetic models.[48] Of the 47 metabolites in module 1, only 13 correlated significantly with BMI at diagnosis, suggesting associations of the remaining 34 metabolites with BMI trajectory may be related to the ALS disease process.

The second largest module, module 2, mostly contained primary and secondary bile acids, which generally associated with the increase BMI trajectory, in addition to metabolites of methionine, cysteine, S-adenosyl methionine, and taurine metabolism and oxidative phosphorylation. Nearly half of the metabolites in this module also correlated significantly with diagnosis BMI (13 species). Bile acids play important roles in nutrient absorption, regulation of cholesterol metabolism, and systemic energy expenditure,[49] so the correlation with BMI trajectory herein is unsurprising. Interestingly, although not present in the module, two bile acids, ursodeoxycholic and its taurine derivative tauroursodeoxycholic acid (taurursodiol), showed some efficacy in ALS clinical trials.[50–53]

Module 3 contained modified amino acids and nucleotide derivatives spanning 22 species evenly split between the decrease and increase BMI groups, of which 9 correlated significantly with diagnosis BMI. Module 4 contained several bioactive lipids, plasmalogens (10 species), lyso-plasmalogens (3 species), and phosphatidylcholines (2 species), which associated mostly with the decrease BMI group, that is, poorer survival. Only two species were significantly linked with diagnosis BMI. We[26] and others[42,45,54,55] have shown that phosphatidylcholines differentiate ALS from control participants, in particular phosphatidylcholine 36:4.[45,54]

Modules 5 and 6 comprised candidates related to energy metabolism. Module 5 contained four short-chain acyl-carnitines intermediates, which all save one correlated with the decrease BMI group. We previously reported acyl-carnitines, along with free fatty acids, contributed to the discrimination between ALS vs control participants,[25,26] which we attributed to either dysfunctional or at-capacity β-oxidation.[56] Modules 6, 7, and 8 contained few metabolites equally divided in their correlation with either the decrease or increase BMI trajectory group, suggesting ALS status may be a stronger determinant of these metabolites than BMI trajectory.

Overall, across some modules, such as module 5, there were more metabolites from various biochemical pathways relating to energy utilization (eg, fatty acid β-oxidation) that are more discerning of ALS vs control participants than of BMI trajectories. These findings suggest that ALS status is a major determinant of energy metabolism. One possibility is that metabolites correlate with fat mass loss in ALS patients,[57] an idea supported by studies where ALS polygenic risk associates with body fat percentage in addition to BMI.[58,59] Interestingly, neither creatine nor creatinine were among the metabolites correlating with BMI change or diagnosis BMI, indicating weight changes may be more pronounced for fat mass than muscle mass. However, lacking body composition measures, we could not evaluate this possibility in this study.

This study has limitations. Participants self-reported weight, potentially incurring recall bias; however, Lin's concordance correlation coefficient was high for participants with available weight, indicating good recall. Our study did not query weight at frequent intervals, so we cannot determine whether BMI loss in ALS participants was linear in the 5 years before study entry or more pronounced closer to diagnosis. It is also possible we failed to detect an onset in BMI changes between the 10- to 5-year window before diagnosis due to the lack of granular BMI information. Next, we only asked participants to report current height, and use this for BMI calculations at all time-points. However, such changes in height over the life course are not anticipated to cause bias in statistical models.[60] We also did not collect a dietary or physical activity survey for this analysis. Additionally, our metabolomics analysis was untargeted, and thus did not measure all metabolites in every relevant biochemical pathway. Although BMI analysis was longitudinal, metabolomics analysis was cross-sectional. Plasma samples for untargeted metabolomics were nonfasted for ethical reasons, as noted in our earlier publications.[25,26]

In conclusion, we found that ALS participants have distinct BMI trajectories vs controls, with the most significant BMI drop occurring within 5 years before diagnosis. ALS participants with normal baseline BMI and decrease BMI trajectory, or baseline obese BMI and increase BMI trajectory, have shorter survival. BMI trajectories correlate with metabolic changes, especially with sphingomyelins and bile acids.

AUTHOR CONTRIBUTIONS

Stephen Aaron Goutman: Conceptualization; data curation; funding acquisition; investigation; project administration; writing – original draft; writing – review and editing. **Jonathan Boss:** Formal analysis; writing – review and editing. **Gayatri Iyer:** Formal analysis; writing – review and editing. **Hani Habra:** Formal analysis; writing – review and editing. **Masha Savelieff:** Writing – review and editing. **Alla Karnovsky:** Formal analysis; writing – review and editing. **Bhramar Mukherjee:** Conceptualization; formal analysis; writing – review and editing. **Eva L Feldman:** Funding acquisition; investigation; project administration; resources; supervision; writing – review and editing.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

Deidentified data may be made available to qualified investigators upon reasonable request.

ETHICAL PUBLICATION STATEMENT

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ORCID

Stephen A. Goutman https://orcid.org/0000-0001-8780-6637
Jonathan Boss https://orcid.org/0000-0001-5285-3038
Gayatri Iyer https://orcid.org/0000-0002-8100-0832
Hani Habra https://orcid.org/0000-0003-4838-0105
Masha G. Savelieff https://orcid.org/0000-0001-5575-2494
Alla Karnovsky https://orcid.org/0000-0001-7388-8520
Bhramar Mukherjee https://orcid.org/0000-0003-0118-4561
Eva L. Feldman https://orcid.org/0000-0002-9162-2694

REFERENCES

1. Goutman SA, Hardiman O, Al-Chalabi A, et al. Recent advances in the diagnosis and prognosis of amyotrophic lateral sclerosis. *Lancet Neurol*. 2022;21(5):480-493. doi:10.1016/S1474-4422(21)00465-8

2. Goutman SA, Hardiman O, Al-Chalabi A, et al. Emerging insights into the complex genetics and pathophysiology of amyotrophic lateral sclerosis. *Lancet Neurol*. 2022;21(5):465-479. doi:10.1016/S1474-4422(21)00414-2

3. Benatar M, Turner MR, Wuu J. Defining pre-symptomatic amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener*. 2019;20(5–6):303-309. doi:10.1080/21678421.2019.1587634

4. Moglia C, Calvo A, Grassano M, et al. Early weight loss in amyotrophic lateral sclerosis: outcome relevance and clinical correlates in a population-based cohort. *J Neurol Neurosurg Psychiatry*. 2019;90(6):666-673. doi:10.1136/jnnp-2018-319611

5. Peter RS, Rosenbohm A, Dupuis L, et al. Life course body mass index and risk and prognosis of amyotrophic lateral sclerosis: results from the ALS registry Swabia. *Eur J Epidemiol*. 2017;32(10):901-908. doi:10.1007/s10654-017-0318-z

6. Nakken O, Meyer HE, Stigum H, Holmoy T. High BMI is associated with low ALS risk: a population-based study. *Neurology*. 2019;93(5):e424-e432. doi:10.1212/WNL.0000000000007861

7. O'Reilly ÉJ, Wang H, Weisskopf MG, et al. Premorbid body mass index and risk of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener*. 2013;14(3):205-211. doi:10.3109/21678421.2012.735240

8. O'Reilly EJ, Wang M, Adami HO, et al. Prediagnostic body size and risk of amyotrophic lateral sclerosis death in 10 studies. *Amyotroph Lateral Scler Frontotemporal Degener*. 2018;19(5–6):396-406. doi:10.1080/21678421.2018.1452944

9. Mariosa D, Beard JD, Umbach DM, et al. Body mass index and amyotrophic lateral sclerosis: a study of US military veterans. *Am J Epidemiol*. 2017;185(5):362-371. doi:10.1093/aje/kww140

10. Gallo V, Wark PA, Jenab M, et al. Prediagnostic body fat and risk of death from amyotrophic lateral sclerosis: the EPIC cohort. *Neurology*. 2013;80(9):829-838. doi:10.1212/WNL.0b013e3182840689

11. Ioannides ZA, Ngo ST, Henderson RD, McCombe PA, Steyn FJ. Altered metabolic homeostasis in amyotrophic lateral sclerosis: mechanisms of energy imbalance and contribution to disease progression. *Neurodegener Dis*. 2016;16(5–6):382-397. doi:10.1159/000446502

12. Steyn FJ, Ioannides ZA, van Eijk RPA, et al. Hypermetabolism in ALS is associated with greater functional decline and shorter survival. *J Neurol Neurosurg Psychiatry*. 2018;89(10):1016-1023. doi:10.1136/jnnp-2017-317887

13. Dupuis L, Pradat PF, Ludolph AC, Loeffler JP. Energy metabolism in amyotrophic lateral sclerosis. *Lancet Neurol*. 2011;10(1):75-82. doi:10.1016/S1474-4422(10)70224-6

14. Mariosa D, Hammar N, Malmstrom H, et al. Blood biomarkers of carbohydrate, lipid, and apolipoprotein metabolisms and risk of amyotrophic lateral sclerosis: a more than 20-year follow-up of the Swedish AMORIS cohort. *Ann Neurol*. 2017;81(5):718-728. doi:10.1002/ana.24936

15. Ingre C, Chen L, Zhan Y, Termorshuizen J, Yin L, Fang F. Lipids, apolipoproteins, and prognosis of amyotrophic lateral sclerosis. *Neurology*. 2020;94(17):e1835-e1844. doi:10.1212/WNL.0000000000009322

16. Cirulli ET, Guo L, Leon Swisher C, et al. Profound perturbation of the metabolome in obesity is associated with health risk. *Cell Metab*. 2019;29(2):488-500.e2. doi:10.1016/j.cmet.2018.09.022

17. Ho JE, Larson MG, Ghorbani A, et al. Metabolomic profiles of body mass index in the Framingham heart study reveal distinct cardiometabolic phenotypes. *PLoS One*. 2016;11(2):e0148361. doi:10.1371/journal.pone.0148361

18. Kraus WE, Pieper CF, Huffman KM, et al. Association of plasma small-molecule intermediate metabolites with age and body mass index across six diverse study populations. *J Gerontol A Biol Sci Med Sci*. 2016;71(11):1507-1513. doi:10.1093/gerona/glw031

19. Goutman SA, Boss J, Patterson A, Mukherjee B, Batterman S, Feldman EL. High plasma concentrations of organic pollutants negatively impact survival in amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatry*. 2019;90(8):907-912. doi:10.1136/jnnp-2018-319785

20. Su FC, Goutman SA, Chernyak S, et al. Association of environmental toxins with amyotrophic lateral sclerosis. *JAMA Neurol*. 2016;73(7):803-811. doi:10.1001/jamaneurol.2016.0594

21. Yu Y, Su FC, Callaghan BC, Goutman SA, Batterman SA, Feldman EL. Environmental risk factors and amyotrophic lateral sclerosis (ALS): a case-control study of ALS in Michigan. *PloS One*. 2014;9(6):e101186. doi:10.1371/journal.pone.0101186

22. Goutman SA, Boss J, Godwin C, Mukherjee B, Feldman EL, Batterman SA. Associations of self-reported occupational exposures and settings to ALS: a case-control study. *Int Arch Occup Environ Health*. 2022;95(7):1567-1586. doi:10.1007/s00420-022-01874-4

23. Brooks BR, Miller RG, Swash M, Munsat TL, World Federation of Neurology Research Group on Motor Neuron D. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord*. 2000;1(5):293-299. doi:10.1080/146608200300079536

24. Keys A, Fidanza F, Karvonen MJ, Kimura N, Taylor HL. Indices of relative weight and obesity. *J Chronic Dis*. 1972;25(6):329-343. doi:10.1016/0021-9681(72)90027-6

25. Goutman SA, Boss J, Guo K, et al. Untargeted metabolomics yields insight into ALS disease mechanisms. *J Neurol Neurosurg Psychiatry*. 2020;91(12):1329-1338. doi:10.1136/jnnp-2020-323611

26. Goutman SA, Guo K, Savelieff MG, et al. Metabolomics identifies shared lipid pathways in independent amyotrophic lateral sclerosis cohorts. *Brain J Neurol*. 2022;awac025. doi:10.1093/brain/awac025

27. Liang KY, Zeger SL. Longitudinal data-analysis using generalized linear-models. *Biometrika*. 1986;73(1):13-22. doi:10.1093/biomet/73.1.13

28. Halekoh U, Højsgaard S, Yan J. The R package geepack for generalized estimating equations. *J Stat Softw*. 2006;15(2):1-11. doi:10.18637/jss.v015.i02

29. Genolini C, Alacoque X, Sentenac M, Arnaud C. kml and kml3d: R packages to cluster longitudinal data. *J Stat Softw*. 2015;65(4):34. doi:10.18637/jss.v065.i04

30. Calinski T, Harabasz J. A dendrite method for cluster analysis. *Commun Stat Theory Methods*. 1974;3(1):1-27. doi:10.1080/03610927408827101

31. Gower JC. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika*. 1966;53(3/4):325. doi:10.1093/biomet/53.3-4.325

32. Basu S, Duren W, Evans CR, Burant CF, Michailidis G, Karnovsky A. Sparse network modeling and metscape-based visualization methods for the analysis of large-scale metabolomics data. *Bioinformatics*. 2017;33(10):1545-1553. doi:10.1093/bioinformatics/btx012

33. Iyer GR, Wigginton J, Duren W, et al. Application of differential network enrichment analysis for deciphering metabolic alterations. *Metabolites*. 2020;10(12):479. doi:10.3390/metabo10120479

34. Ma J, Karnovsky A, Afshinnia F, et al. Differential network enrichment analysis reveals novel lipid pathways in chronic kidney disease. *Bioinformatics*. 2019;35(18):3441-3452. doi:10.1093/bioinformatics/btz114

35. Lancichinetti A, Fortunato S. Consensus clustering in complex networks. *Sci Rep*. 2012;2(1):336. doi:10.1038/srep00336

36. Friedman J, Hastie T, Tibshirani R. A note on the group lasso and a sparse group lasso. *arXiv*. 2010;22:10010736.

37. Huisman MH, Seelen M, van Doormaal PT, et al. Effect of presymptomatic body mass index and consumption of fat and alcohol on amyotrophic lateral sclerosis. *JAMA Neurol*. 2015;72(10):1155-1162. doi:10.1001/jamaneurol.2015.1584

38. Westeneng HJ, van Veenhuijzen K, van der Spek RA, et al. Associations between lifestyle and amyotrophic lateral sclerosis stratified by C9orf72 genotype: a longitudinal, population-based, case-control study. *Lancet Neurol*. 2021;20(5):373-384. doi:10.1016/S1474-4422(21)00042-9

39. Janse van Mantgem MR, van Eijk RPA, van der Burgh HK, et al. Prognostic value of weight loss in patients with amyotrophic lateral sclerosis: a population-based study. *J Neurol Neurosurg Psychiatry*. 2020;91(8):867-875. doi:10.1136/jnnp-2020-322909

40. Nakamura R, Kurihara M, Ogawa N, et al. Prognostic prediction by hypermetabolism varies depending on the nutritional status in early amyotrophic lateral sclerosis. *Sci Rep*. 2021;11(1):17943. doi:10.1038/s41598-021-97196-5

41. Jésus P, Fayemendy P, Nicol M, et al. Hypermetabolism is a deleterious prognostic factor in patients with amyotrophic lateral sclerosis. *Eur J Neurol*. 2018;25(1):97-104. doi:10.1111/ene.13468

42. Bjornevik K, Zhang Z, O'Reilly ÉJ, et al. Prediagnostic plasma metabolomics and the risk of amyotrophic lateral sclerosis. *Neurology*. 2019;92(18):e2089-e2100. doi:10.1212/wnl.0000000000007401

43. Lawton KA, Brown MV, Alexander D, et al. Plasma metabolomic biomarker panel to distinguish patients with amyotrophic lateral sclerosis

from disease mimics. *Amyotroph Lateral Scler Frontotemporal Degener.* 2014;15(5–6):362-370. doi:10.3109/21678421.2014.908311

44. Lawton KA, Cudkowicz ME, Brown MV, et al. Biochemical alterations associated with ALS. *Amyotroph Lateral Scler.* 2012;13(1):110-118. doi:10.3109/17482968.2011.619197

45. Sol J, Jové M, Povedano M, et al. Lipidomic traits of plasma and cerebrospinal fluid in amyotrophic lateral sclerosis correlate with disease progression. *Brain Commun.* 2021;3(3):fcab143. doi:10.1093/braincomms/fcab143

46. Cutler RG, Pedersen WA, Camandola S, Rothstein JD, Mattson MP. Evidence that accumulation of ceramides and cholesterol esters mediates oxidative stress-induced death of motor neurons in amyotrophic lateral sclerosis. *Ann Neurol.* 2002;52(4):448-457. doi:10.1002/ana.10312

47. Wang G, Bieberich E. Sphingolipids in neurodegeneration (with focus on ceramide and S1P). *Adv Biol Regul.* 2018;70:51-64. doi:10.1016/j.jbior.2018.09.013

48. Mohassel P, Donkervoort S, Lone MA, et al. Childhood amyotrophic lateral sclerosis caused by excess sphingolipid synthesis. *Nat Med.* 2021;27(7):1197-1204. doi:10.1038/s41591-021-01346-1

49. Di Ciaula A, Garruti G, Lunardi Baccetto R, et al. Bile acid physiology. *Ann Hepatol.* 2017;16(Suppl. 1: s3–105):s4-s14. doi:10.5604/01.3001.0010.5493

50. Paganoni S, Macklin EA, Hendrix S, et al. Trial of sodium Phenylbutyrate-Taurursodiol for amyotrophic lateral sclerosis. *N Engl J Med.* 2020;383(10):919-930. doi:10.1056/NEJMoa1916945

51. Paganoni S, Hendrix S, Dickson SP, et al. Long-term survival of participants in the CENTAUR trial of sodium phenylbutyrate-taurursodiol in amyotrophic lateral sclerosis. *Muscle Nerve.* 2021;63(1):31-39. doi:10.1002/mus.27091

52. Parry GJ, Rodrigues CM, Aranha MM, et al. Safety, tolerability, and cerebrospinal fluid penetration of ursodeoxycholic acid in patients with amyotrophic lateral sclerosis. *Clin Neuropharmacol.* 2010;33(1):17-21. doi:10.1097/WNF.0b013e3181c47569

53. Min JH, Hong YH, Sung JJ, Kim SM, Lee JB, Lee KW. Oral solubilized ursodeoxycholic acid therapy in amyotrophic lateral sclerosis: a randomized cross-over trial. *J Korean Med Sci.* 2012;27(2):200-206. doi:10.3346/jkms.2012.27.2.200

54. Blasco H, Veyrat-Durebex C, Bocca C, et al. Lipidomics reveals cerebrospinal-fluid signatures of ALS. *Sci Rep.* 2017;7(1):17652. doi:10.1038/s41598-017-17389-9

55. Chang KH, Lin CN, Chen CM, et al. Altered metabolic profiles of the plasma of patients with amyotrophic lateral sclerosis. *Biomedicine.* 2021;9(12):1944. doi:10.3390/biomedicines9121944

56. van Eunen K, Simons SM, Gerding A, et al. Biochemical competition makes fatty-acid β-oxidation vulnerable to substrate overload. *PLoS Comput Biol.* 2013;9(8):e1003186. doi:10.1371/journal.pcbi.1003186

57. Lee I, Kazamel M, McPherson T, et al. Fat mass loss correlates with faster disease progression in amyotrophic lateral sclerosis patients: exploring the utility of dual-energy x-ray absorptiometry in a prospective study. *PLoS One.* 2021;16(5):e0251087. doi:10.1371/journal.pone.0251087

58. Li C, Ou R, Wei Q, Shang H. Shared genetic links between amyotrophic lateral sclerosis and obesity-related traits: a genome-wide association study. *Neurobiol Aging.* 2021;102:211 e1-211 e9. doi:10.1016/j.neurobiolaging.2021.01.023

59. Zhang L, Tang L, Huang T, Fan D. Life course adiposity and amyotrophic lateral sclerosis: a Mendelian randomization study. *Ann Neurol.* 2020;87(3):434-441. doi:10.1002/ana.25671

60. Fernihough A, McGovern ME. Physical stature decline and the health status of the elderly population in England. *Econ Hum Biol.* 2015;16:30-44. doi:10.1016/j.ehb.2013.12.010

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.